

Three-Dimensional Reconstruction of Promastigote of *Leishmania Amazonensis* Treated With ACET-1 from Serial Sections Obtained by FIB-SEM

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Leishmaniasis comprises a group of diseases that are caused by parasites the genus *Leishmania*, presented as a severe public health problem in many countries worldwide, affecting mainly regions that are socially and economically vulnerable^[1]. The chemotherapeutic arsenal comprises just three main drugs (pentavalent antimonials, amphotericin B and miltefosine), which exhibit several limitations, including long administration regimens, hospitalization, high costs and severe adverse effects. The search for new drugs therefore remains a necessity, especially for developing countries ^[2], however, in order to succeed in the development these new drugs, it is necessary to know all the structural and functional biology of the parasite, for the purpose of identify possible therapeutic targets. In this context, our research group has already shown in previous studies, that the synthetic compound 4-(5'-formyl-[2,2'-bithiophen]-5-yl)but-3-yn-1-yl acetate, **ACET-1**, induced ultrastructural and morphological changes in promastigotes of *Leishmania amazonensis*, when treated with IC₅₀ (28.9 μM) for 72 h. Based on this, we propose a strategy to increase the understanding of the mechanism of action of this compound, mainly on the cytoskeleton and plasma membrane of promastigote, and consequently the understanding of the ultrastructure of the parasite, using FIB-SEM techniques and producing a virtual 3D models. For this, promastigote (1 x 10⁶ cells ml⁻¹) were treated with IC₅₀ of **ACET-1** for 72 h at 28 °C. After, the parasites were fixed in a solution of 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 24 h at 4 °C. Past this time, the parasites were post-fixed in a solution of 1% OsO₄, 0.8% potassium ferricyanide and 10 mM CaCl₂ in 0.1 M cacodylate buffer. The samples were dehydrated in increasing acetone gradient and embedded in Polybed 812 resin. Sputter-coated with gold was performed and the serial sections were obtained using the FIB-SEM double-beam microscope (FEI Scios). The alignment, segmentation and cell rendering was realized by AMIRA (FEI Company, USA). Our results demonstrated that **ACET-1** induced morphological and ultrastructural alterations in promastigote at molecular level, destabilizing the protein bonds between the subpellicular microtubules and the plasma membrane resulting in morphological alterations.

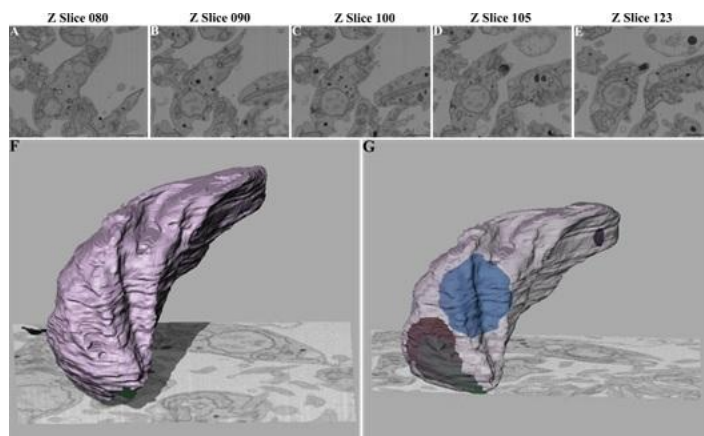


Figure 1: 3D reconstruction of the whole volume of promastigote of *L. amazonensis* by serial section. (A-D) Obtainment of serial images from 20 nm – thick serial sections. (E) 3D model showing the plasma membrane (lightpink). (F) 3D model showing the cell nucleus (blue), kinetoplast (red), flagellum (green) and lipids bodies (purple). (G) SEM image. Scale bar: (A-D): 3 μm ; (G): 5 μm ;

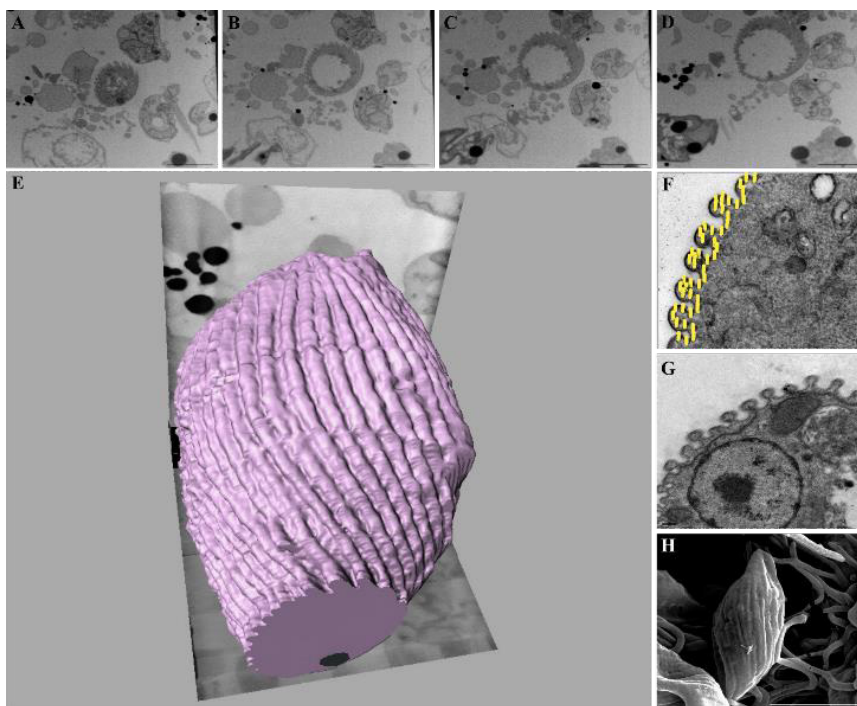


Figure 2: 3D reconstruction of the whole volume of promastigote of *L. amazonensis* treated with IC_{50} of ACET-1 by serial section. (A-D) Obtainment of serial images from 20 nm – thick serial sections. (E) 3D model showing the plasma membrane (lightpink). (F) 3D model showing microtubules (yellow). (G) TEM image. (H) SEM image. Scale bar: (A-D): 4 μm ; (G): 0,3 μm ; (H): 3 μm ;

References:

- [1] P. David., Journal International of Information Security **69** (2014), p. 10-18.
- [2] T. Neeraj., et al. Mini-Reviews in Medicinal Chemistry **18** (2017), p 26-41.
- [3]The authors acknowledge funding from the COMCAP-UEM, CNPq, CAPES, PRONEX/Fundação Araucária, FINEP.